Editorial

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NKG2D and its ligands: Key to immunotherapy of liver cancer?

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Hepatocellular carcinoma (HCC) is a common cancer accounting for a significant proportion of all cancers worldwide. Although most cases of HCC occur in countries where hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are endemic, the tumour is ubiquitous and one of the most challenging complications of advanced liver disease. HCC develops deviously and there are currently no reliable biomarkers for early diagnosis. Indeed, because alpha-foetoprotein (AFP) has poor sensitivity and specificity, and other serological markers such as des-gamma-carboxyprothrombin (DCP), and *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) perform only slightly better [1], abdominal ultrasound is presently considered the tool of choice for surveillance of patients at risk. Treatment of HCC has considerably improved over the last few decades from mere surgical resection in a minority of patients with good hepatic function and small tumours to orthotopic liver transplantation in selected patients satisfying the stringent Milan criteria [2], to locoregional ablation procedures, transarterial chemoembolization, and more recently antiangiogenetic drugs that increase survival in patients with advanced HCC for whom no therapy was hitherto available. Targeted treatment options are expected to expand exponentially as specific molecular signatures are being identified for HCC subgroups defined by microarray analysis [3]. Despite the wealth of information on molecular biology, tumour growth rate, surveillance, diagnosis and management, there is currently only a scarcity of seminal studies addressing the immunopathogenesis of HCC, which may have important implications in the design of immunotherapeutic strategies. With respect to adaptive immunity it has been suggested that antigens such as AFP, the melanoma-associated antigen (MAGE), glypican 3 and NY-ESO, which are highly expressed in HCC (reviewed in [4]), are potential targets for T-cell responses. Moreover, several studies suggested that the presence of tumour-infiltrating cytotoxic T-cells is indicative of better survival. Interestingly, treatment of neoplastic nodules with radiofrequency thermal ablation (RFTA) can enhance the release and exposure of tumour antigens, which might help to overcome immune tolerance towards cancer cells [5]. Innate immune responses have only been marginally explored in the setting of HCC. Natural killer (NK) cells are an essential component of innate immunity being instrumental in anti-tumour immune responses [6]. A recent study provided important evidence in favour of their role in HCC, with increased frequencies of NK cells expressing higher levels of activating and reduced levels of inhibitory NK receptors, together with increased functional activity, e.g. interferon-γ production and cytotoxicity, in patients treated with RFTA [7]. Interestingly, recurrence-free survival correlated with sustained functional NK cell activation, suggesting a role for these cells in the control of liver cancer.

In this issue of the *Journal*, Kamimura *et al.* take a fresh look at the role of NK cells in HCC by examining correlates of natural-killer group 2, member D (NKG2D) ligand expression and tumour recurrence. NKG2D is a fundamental activating receptor belonging to the C-type lectin-like family which is constitutively expressed on NK cells, most NKT cells, some $\gamma\delta$T and CD8 T cells (reviewed in [8]), and unlike other NKG2 receptors, does not associate with CD94. The seemingly invariant activating receptor NKG2D promiscuously binds to multiple ligands such as major histocompatibility complex class I-related chain A and B (MICA/B) and the unique long 16 (UL16)-binding protein family (ULBPs) that are poorly, if at all expressed on healthy cells but they are up-regulated by stress, viral infection or DNA damage [9]. Up-regulation of these ligands may tip the balance of NK cells from inhibition to activation ("induced self" recognition) with obvious biological implications. The authors show that one of the major NKG2D ligands, ULBP1, was not expressed on poorly differentiated human HCC tissue and on a similarly poorly differentiated HCC cell line, whereas it was abundantly expressed on dysplastic nodules and well to moderately differentiated HCC. Moreover, loss of ULBP1 expression was not related to reduced mRNA expression suggesting that post-transcriptional events were responsible for it. Indeed, evidence in support of their hypothesis came from proteasome inhibition experiments which resulted in upregulation of ULBP1. More importantly, recurrence-free survival, even though not the overall survival, was significantly shorter in patients with ULBP1-negative tumours and loss of ULBP1 was an independent predictor of early recurrence. These findings provide corroborative evidence in favour of a role of NK cells and, more specifically of the NKG2D receptor pathway, in liver cancer immune surveillance.

A complex interplay between NKG2D and its ligands may be involved in the natural history and response to treatment in a variety of cancers, including HCC [8,10]. However, although

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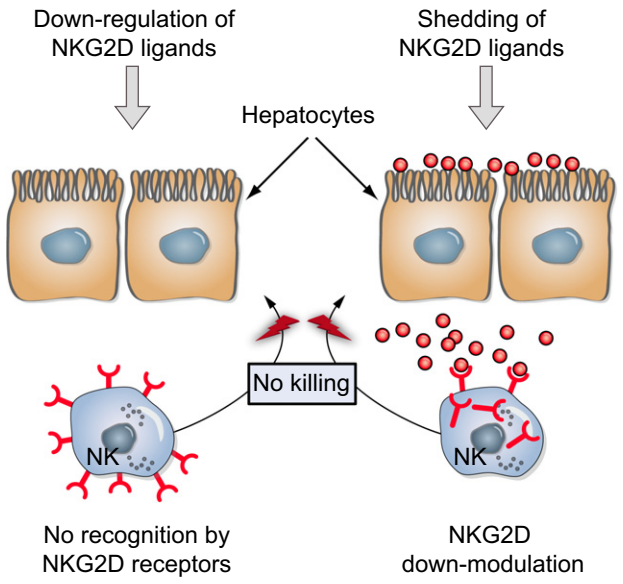

Fig. 1. Mechanisms of inhibition of NK cell killing of tumour cells via the NKG2D receptor/ligand(s) pathway. Inhibition can occur by down-regulation of ligand(s) on target cells or by down-modulation of NKG2D on effector cells via shed ligand(s).

NKG2D ligand expression on cancer cells is generally associated with indolent disease progression and prolonged survival in several types of tumour [11], increased ligand shedding may be one mechanism responsible for NKG2D down-regulation, decreased cytotoxic potential and tumour progression, at least when soluble MICA is released in excess by neoplastic cells, as shown in some oncological settings including HCC [12–14]. Alternatively, as described by Kamimura, down-regulation of ligands such as ULBP1 may prevent effective NKG2D killing and promote cancer recurrence (Fig. 1). An additional aspect of the potential importance of the subversion of the NKG2D functional pathway pertains to chronic hepatitis C virus (HCV) infection which is responsible for cirrhosis and HCC in the majority of patients in the Western world and Japan. Indeed, it has recently been shown that the HCV NS5A protein stimulates the production of IL-10 which in turn triggers the secretion of TGF-β that down-modulates NKG2D expression on NK cells [15]. This may have further impact on the subgroup of patients developing HCC in the context of a chronic HCV infection.

Although NKG2D-ligand interaction appears to be an important pathway of cancer control mediated by NK cells, the expression of several other ligands of activating and inhibitory NK receptors may also play a role in controlling liver carcinogenesis. For the former, DNAM-1, which has been shown to play a role together with NKG2D in NK killing of Ewing's sarcoma cells [16] has been acknowledged to potentially be involved in HCC [17]: This is particularly relevant to Kamimura's study, since NK cell degranulation could not be effectively inhibited by anti-ULBP1 blockade. However, antibody blocking is usually not as effective as silencing with specific siRNAs and this may, to some extent, have influenced the data. Nonetheless, it would be naïve to ignore the role of other activating receptors such as NKp30, NKp44, and NKp46, although their ligands are far less defined than those of NKG2D [18]. Furthermore, the role of HLA/killer immunoglobulin-like inhibitory receptors (KIR) match, has been largely overlooked. To this end, it may be inferred that individuals homozygous for weak KIR-ligand combinations are more likely to counteract proliferation of HCC, since NK cell inhibition is more easily overcome in patients with weak KIR-ligand binding, allowing more effective lysis of tumour cells.

It is now time that immunotherapy of liver cancer be rescued from neglect or experimental stage in animal models. Growing evidence indicate that manipulation of innate immune responses could be a viable option and one possibility would be to activate NK cells *ex vivo* to upregulate NKG2D (or other activating receptors), to induce ligand expression on tumour tissue *in vivo*, or both. Infusion of activated allogeneic NK cells, which do not require identification of tumour antigens, antigen priming, or vaccination strategies and do not cause graft *vs.* host disease, should be considered for proof-of-concept studies of HCC immunotherapy. Recent studies suggest that proteasome inhibitors, such as bortezomib or MG132 may render HCC and other tumours more susceptible to NKG2D- (as also shown here) or tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cell lysis [19]. This may represent a valid application of adjuvant immunotherapy for patients treated with locoregional ablative therapies or with chemotherapeutic agents able to sensitize HCC cells to NK lysis. To this end, sorafenib has been shown to reduce MICA shedding, which inhibits NKG2D-mediated killing [20]. A better understanding of NK cell-mediated HCC immune surveillance will help in the design of innovative therapeutic approaches in a field that has for long relied on rather primitive treatment aids. The stakes are too high for us to afford to fail the challenge.

Conflict of interest

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